Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Reports of in-house investigations of **Cholic Acid** (missing), **Pyridoxine Hydrochloride**, **Dextran** & **Potassium Sulfate** in the developing chicken embryos 7/17/78

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Hearing Clerk, HFC-20

DATE: 17 JUL 1978

FROM : Leo F. Mansor, HFF-335
GRAS Review Branch

SUBJECT: Chicken Embryo Study Reports

Enclosed are reports of the inhouse investigations in the developing chicken embryo on the following substances:

1. Pyridoxine Hydrochloride
2. Dextran
3. Potassium sulfate
4. Cholic Acid

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335 DATE: June 16, 1978

THRU: HFF-150 _____

FROM : Team Leader, Chicken Embryo Research Team
Whole Animal Toxicology Branch, HFF-155

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Potassium Sulfate in the developing chicken embryo.

M. Jacqueline Verrett

M. Jacqueline Verrett, Ph.D.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Potassium Sulfate

Protocol:

Potassium Sulfate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Column 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Potassium sulfate, nap, very little toxicity when administered via the air cell, and no LD_{50} could be calculated for either time of administration. Yolk treatment at 0 hours resulted in a calculated LD_{50} of 100±15 mg/kg (5.01 mg/egg); the regression line for yolk treatment at 96 hours had a slope that was not significantly different from zero ($p = 0.05$).

Scattered abnormalities were observed for all four conditions of test, but they were not different from or significantly higher than that observed in the solvent-treated or untreated controls. Potassium sulfate displayed no teratogenicity up to 200 mg/kg under the test conditions employed.

1. Potassium Sulfate, Lot # 618-8696, Baker Analyzed Reagents

2. McLaughlin, J., Jr., Marliac, J. P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770

3. Verrett, M. J., Marliac, J.P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006

4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Potassium Sulfate
Air Cell at 0 Hours

Table 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	120	33.33	4.16	2.50
5.00	100.00	120	35.00	2.50	0.83
2.50	50.00	120	39.16	1.66	0.00
1.250	25.00	120	37.50	5.00	0.83
0.500	10.00	120	38.33	1.66	0.83
Water		120	42.50	2.50	0.00
Controls		343	13.41	1.16	0.87

**Slope is negative

Potassium Sulfate
Air Cell at 96 Hours

Table 2

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	115	49.56*	4.34	1.73
2.50	50.00	115	36.52	6.95*	1.73
1.250	25.00	114	25.43	0.87	0.00
0.6250	12.50	113	21.23	7.96*	1.76
0.250	5.00	114	33.33	3.50	1.75
Water		120	33.33	0.00	0.00
Controls		343	13.41	1.16	0.87

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$

Potassium Sulfate
Yolk at 0 Hours

Table 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	120	85.00*	1.66	1.66
5.00	100.00	120	77.50*	0.83	0.83
2.50	50.00	119	80.67*	1.68	1.68
1.250	25.00	120	71.66	3.33	1.66
0.500	10.00	119	68.90	0.84	0.00
Water		120	64.16	0.83	0.00
Controls		343	13.41	1.16	0.87

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 100.1154 mg/kg (5.006 mg/egg)

Potassium Sulfate
Yolk at 96 Hours

Table 4

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	110	61.81*	10.90*	1.81
2.50	50.00	110	65.45*	4.54	0.90
1.250	25.00	110	54.54*	0.90	0.00
0.6250	12.50	110	56.36*	6.36	1.81
0.250	5.00	110	57.27*	4.54	3.63
Water		120	37.50	2.50	1.66
Controls		343	13.41	1.16	0.87

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: June 8, 1978

THRU: HFF-150

FROM : Supervisory Chemist
Whole Animal Toxicology Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigation of

Dextran in the developing chicken embryo.

M. Jacqueline Verrett, Ph.D.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Dextran administered via the air cell produced no toxicity above background up to 200 mg/kg (10 mg/egg). Yolk administration at 0 hours resulted in a calculated LD_{50} of 180.07 mg/kg (9.0 mg/egg). Yolk treatment at 96 hours resulted in slightly elevated toxicity but the slope of the regression line was not significantly different from zero ($p = 0.05$) and no LD_{50} could be calculated.

Scattered abnormalities were observed under all conditions of test, but in no instance were serious abnormalities significantly higher than or different from those observed in the background. Dextran displayed no teratogenicity under the test conditions employed.

1. Dextran Industrial, Grade "R" Lot # R76305, Company Unknown

2. McLaughlin, J., Marliac, J.P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770

3. Verrett, M.J., Marliac, J.P., and McLaughlin, J., Jr., (1964) JOAC 47, 1002-1006

4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Dextran

Protocol:

Dextran (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Dextran
Air Cell at 0 Hours

Table 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	115	21.73	7.82	0.86
5.00	100.00	115	27.82	7.82	0.86
2.50	50.00	115	21.73	1.73	0.86
1.250	25.00	114	27.19	1.75	0.00
0.500	10.00	114	19.29	0.87	0.00
Water		115	30.43	1.73	0.00
Controls		303	22.11	2.31	0.33

**Slope not significantly different from zero p = 0.05

Dextran
Air Cell at 96 Hours

Table 2

Dose mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	110	36.36	2.72	0.90
2.50	50.00	110	30.00	0.90	0.90
1.250	25.00	110	40.90	7.27*	3.63
0.6250	12.50	109	49.54	4.58	3.66
0.250	5.00	109	42.20	1.83	0.00
Water		110	37.27	0.90	0.00
Controls		303	22.11	2.31	0.33

*Significantly different from solvent $p \leq 0.05$
**Slope is negative

Dextran
Yolk at 0 Hours

Table 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	115	67.82*	1.73	0.00
5.00	100.00	115	69.95*	0.00	0.00
2.50	50.00	115	65.21*	3.47	0.00
1.250	25.00	115	59.13*	0.86	0.86
0.500	10.00	115	51.30	4.34	0.00
Water		115	31.13	3.47	0.00
Controls		303	22.11	2.31	0.33

*Significantly different from solvent $p \leq 0.05$
**LD_{50} 180.0666 mg/kg (9.003 mg/egg)

Dextran
Yolk at 96 Hours

Table 4

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	110	66.36*	1.81	0.90
2.50	50.00	109	61.46*	1.83	0.00
1.250	25.00	110	70.00*	3.63	0.90
0.6250	12.50	110	60.90*	0.90	0.90
0.250	5.00	109	59.63*	0.91	0.91
Water		105	37.14	1.90	0.00
Controls		303	22.11	2.31	0.33

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: June 8, 1978

THRU: HFF-150 _____

FROM : Team Leader, Chicken Embryo Research Team
Whole Animal Toxicology Branch, HFF-155

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substance to the Developing Chicken Embryo

Attached is the report of the inhouse investigations of Pyridoxine Hydrochloride in the developing chicken embryo.

M. Jacqueline Verrett, Ph.D.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Pyridoxine Hydrochloride

Protocol:

Pyridoxine Hydrochloride (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Pyridoxine HCl
Air Cell at 96 Hours

Table 2

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	115	97.39*	2.60	0.00
2.50	50.00	115	83.47*	1.73	0.86
1.250	25.00	115	39.13	11.30*	1.73
0.6250	12.50	115	24.34*	3.47	3.47
0.250	5.00	115	28.69	0.86	0.00
Water		120	40.83	0.83	0.00
Controls		340	11.17	1.47	1.17

*Significantly different from solvent $p \leq 0.05$
**LD_{50} 41.0958 mg/kg (2.055 mg/egg)

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion

Pyridoxine HCl showed no toxicity at up to 200 mg/kg (10 mg/egg) when administered via the air cell at 0 hours. At 96 hours the calculated LD_{50} was 41.096 mg/kg (2.06 mg/egg). Yolk treatment resulted in a calculated LD_{50} of 102.44 mg/kg (5.12 mg/egg). Yolk treatment at 96 hours resulted in a regression line whose slope was not significantly different from zero ($p = 0.05$).

Scattered abnormalities were observed for all four test conditions, but in no instance were the serious abnormalities higher in incidence than or different from those observed in the background. Pyridoxine HCl displayed no teratogenicity under the test conditions employed.

1. Pyridoxine Hydrochloride, Lot #103378, Company Unknown

2. McLaughlin, J., Jr., Marliac, J.P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

3. Verrett, M.J., Marliac, J.P., and McLaughlin, J. Jr., (1964) JAOAC 47, 1002-1006.

4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Pyridoxine HCl
Air Cell at 0 Hours

Table 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	120	38.33	33.33*	3.33
5.00	100.00	119	29.41*	4.20	0.84
2.50	50.00	119	37.81	11.76*	2.52
1.250	25.00	120	30.00	2.50	0.00
0.500	10.00	119	39.49	5.04	0.00
Water		120	42.50	2.50	0.00
Controls		340	11.17	1.47	1.17

*Significantly different from Solvent $p \leq 0.05$

**Slope is negative

Pyridoxine HCl
Yolk at 0 Hours

Table 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	120	87.50*	3.33	0.83
5.00	100.00	119	77.31*	0.84	0.00
2.50	50.00	120	68.33	1.66	1.66
1.250	25.00	119	58.82	2.52	0.00
0.500	10.00	120	55.83	0.00	0.00
Water		120	64.16	0.83	0.00
Controls		340	11.17	1.47	1.17

*Significantly different from zero $p \leq 0.05$
**LD_{50} 102.4378 mg/kg (5.122 mg/egg)

PYRIDOXINE HCL
Yolk at 96 Hours

Table 4

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	114	48.24*	7.89	0.87
2.50	50.00	115	50.43*	0.86	0.00
1.250	25.00	115	52.17*	4.34	0.86
0.6250	12.50	115	39.13	1.73	0.86
0.250	5.00	115	46.08	6.08	4.34
Water		120	35.00	2.50	1.66
Controls		340	11.17	1.47	1.17

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$